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Bureau of Entomology and Plant Quarantine

COLONIZATION OF THE ORGANISM CAUSING MILKY DISEASE

OF JAPANESE BEETLE LARVAE

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The milky diseases of the grubs of the Japanese beetle, caused by certain spore-forming bacteria, have been a major factor in the reduction in numbers of the insect in the areas that have been infested the longest time. Although these diseases are occasionally found in areas only recently infested by the Japanese beetle, the natural spread of the disease organisms tends to lag behind that of the beetle, and it is usually 10 years or more after the beetles reach a given locality before the disease becomes established. In order to accelerate the natural spread and build-up of milky disease, an extensive program of distribution of one of the milky disease organisms, *Bacillus popilliae*, has been undertaken by the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture, in cooperation with a number of State, Federal, and other agencies. The purpose of this report is to make available, to cooperating State workers and others interested, information on the status of this program at the close of the calendar year 1942.

Preparation of Milky Disease Spore Dust Material for Distribution

Milky disease bacteria lend themselves readily to field distribution because of the long life of the spores and their extreme resistance to a wide range of temperature and moisture. As it has not as yet been possible to obtain sporulation of the organisms on artificial media, it has been necessary to propagate the bacteria in the bodies of Japanese beetle grubs in order to get material for field use. One of the most practical means of distributing an organism causing milky disease is to apply it in the form of a dust. Talc, used as the carrier, is impregnated with spores of the organism. This spore-dust mixture is standardized to contain 100 million spores per gram, and thus any desired dosage can be readily applied.

During 1942 some 500,000 grubs were used in the production of the spore dust, of which more than half were furnished by the following agencies: New York Agricultural Experiment Station (Geneva), 100,000; Connecticut Agricultural Experiment Station (New Haven), 90,000; Division of Japanese Beetle Control, 104,000. Three State agencies also inoculated the grubs for most of the spore dust used in their milky disease distribution. These grubs were then processed at the Bureau laboratory at Moorestown, N. J., and the finished dust was returned to the respective State agency for distribution. The total quantities of spore-dust material produced in this manner were as follows: Delaware, 2,208 pounds; Maryland, 10,205 pounds; Ohio, 567 pounds. These quantities were supplemented in some cases by material produced by the Bureau.

Methods of Applying the Spore Dust

A hand corn planter of the rotary type is used in applying the spore dust. The planter can be adjusted to deliver accurately 2 grams (approximately a level teaspoonful) of material per spot, and it holds 1-3/4 pounds, a quantity sufficient to treat 1 acre when applied at that rate at intervals of 10 feet.

Plot studies have shown that where a moderate to heavy Japanese beetle grub population occurs, the milky disease organism will be disseminated throughout a treated area in three seasons if the treated spots are spaced at approximately 10-foot intervals. A much quicker build-up of disease could, of course, be obtained if an area were completely colonized with spore dust, but excessive quantities of material would be needed for such a treatment. The 10-foot-interval treatment has been chosen for the colonization work in most areas.

Distribution Program

The distribution program under way is intended to bring about the establishment of the organism causing milky disease of the Japanese beetle grubs at a large number of locations throughout the entire infested area. The build-up and dispersion of the disease from these centers will then occur at a much faster rate than if natural spread alone were relied upon, and much faster than if a more general treatment were applied to a more limited area. With a program of this type, however, immediate and complete control cannot be expected.

The type of treatment applied has been governed by the specific requirements of the situation involved. Treatment of two half-acre plots per square mile is recommended for open country, and the application is usually made at 10-foot intervals to permanent turf as found in pastures or large lawns. On golf courses, a treatment at 10-foot intervals of approximately one-half acre out of each 10 acres of turf is desirable. In city areas, one block out of each 10 is treated by dusting 10 spots per property (approximately 20 properties or 200 spots per block). In general, this is the equivalent of one-half ^{acres} out of each 10 acres of turf. The general rule on Government reservations is to spot-dust the entire turf area at 10-foot intervals on lines 20 feet apart. This latter type of treatment in places of high grub population gives a rather quick build-up of disease.

A brief discussion of the work conducted throughout the infested area through 1942 is presented in the following pages, and the data are summarized in table 1. Except as previously indicated, the production and processing of the milky disease spore dust were carried on entirely by the Bureau of Entomology and Plant Quarantine. The treatment applications were made chiefly by the cooperating agency, unless it is indicated that the applications were made by the Bureau.

Connecticut.--The Connecticut Agricultural Experiment Station furnished a considerable number of grubs for inoculation and distributed the spore dust in a total of 1,935 sites in 6 counties (approximately 1,320 acres). In addition, the Bureau treated 2 Government reservations involving 88 acres of turf.

Delaware.--The Delaware Agricultural Experiment Station has treated 3,962 individual sites, totaling about 1,981 acres, throughout the 3 counties in this State. The treatments were placed, as far as possible, in permanent pastures, 2 half-acre plots per square mile. A number of towns and cities also received treatment. In addition, one Government reservation comprising 90 acres was treated by the Bureau.

District of Columbia.--In cooperation with the National Capital Park Service, Department of the Interior, the Bureau of Entomology and Plant Quarantine has treated approximately 1,890 acres of turf on Government-owned reservations. In addition, about 4 acres of turf on other properties have received the disease material.

Massachusetts.--In cooperation with the Springfield Department of Public Parks, 6 colony sites of 1 acre each have been treated at 10-foot intervals in Springfield, Hampden County.

Maryland.--Cooperative work between the Bureau of Entomology and Plant Quarantine and the Maryland Agricultural Experiment Station has been in progress since the fall of 1939, and up to December 15, 1942, a total of more than 35,000 colony sites comprising about 20,751 acres in 22 counties and the City of Baltimore had received treatment. In addition to this cooperative program, the Bureau has treated 9 Government reservations comprising 1,238 acres of turf.

New Jersey.--In cooperation with the New Jersey Department of Agriculture, a colonization program involving treatment of 451 colonization sites (323 acres) at approximately 3.5-mile intervals, distributed in all counties of the State except Sussex, has been completed. In addition, 10 Government reservations involving 495 acres were treated by the Bureau of Entomology and Plant Quarantine.

New York.--In the cooperative program the workers of the New York Agricultural Experiment Station and the New York Department of Agriculture and Markets treated 634 locations involving 423 acres in 17 counties. In addition, 1,683 acres of turf in 15 Government reservations were treated by the Bureau.

North Carolina.--Approximately 310 acres were treated at Asheville, in cooperation with the Division of Japanese Beetle Control. Most of the treatment was at 10-foot intervals, with a portion being re-treated at 5-foot intervals. A number of small gardens and pastures were also treated in the Salisbury, East Spencer, and Hendersonville areas.

Ohio.--One hundred and thirty-eight colony sites, approximately 146 acres, in small, local, heavily infested areas, were treated in 7 counties in Ohio in cooperation with the Ohio Agricultural Experiment Station.

Pennsylvania.--In the cooperative program with the Pennsylvania Department of Agriculture, a total of 2,234 sites (1,570 acres) have been treated in 22 counties. Included in the latter are areas located on about 90 golf courses, in which several plots are included under each golf-course site. Four Government reservations totaling 356 acres were also treated by the Bureau.

Rhode Island.--In cooperation with the Rhode Island Department of Agriculture and Conservation, 27 colony sites, involving approximately 40 acres in heavily infested areas throughout 3 counties, have been treated by the Bureau.

Virginia.--Ten Government reservations comprising 372 acres of turf were treated during 1940 and 1941. In the cooperative arrangement with the Virginia Department of Agriculture and Immigration, a total of 742 additional colonization sites involving approximately 413 acres have been treated through 1942 in heavily infested areas in 12 counties.

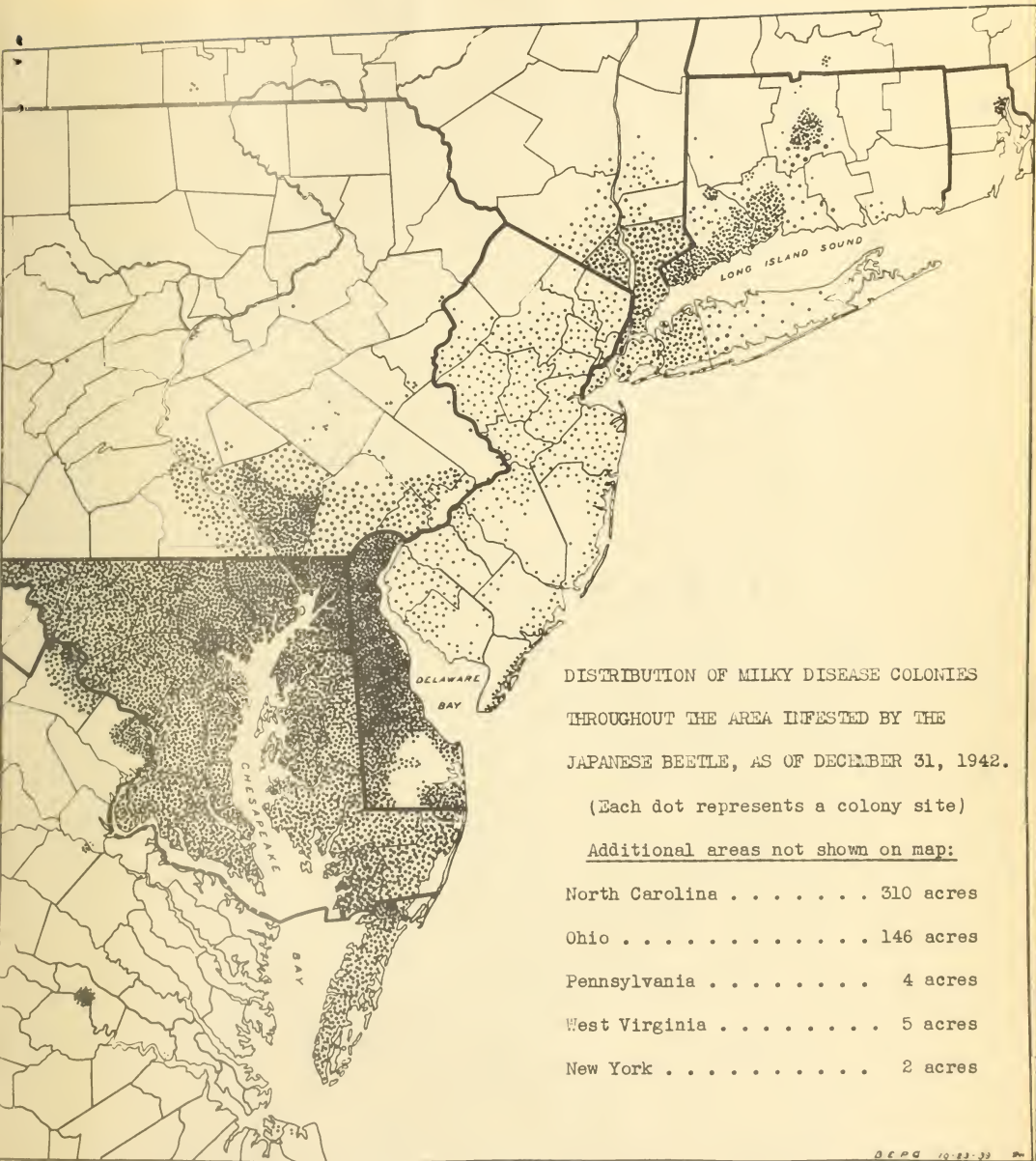
West Virginia.--In cooperation with the West Virginia Department of Agriculture, 8 colony sites involving about 5 acres in 4 counties have been treated.

A total of 33,503 acres in 12 States and the District of Columbia were treated from 1939 through 1942 with over 60,000 pounds of milky disease spore-dust material standardized to contain 100 million spores per gram. An additional 4,000 pounds of spore dust were produced and are ready for distribution during 1943.

The relative distribution of milky disease colonization sites in each of 9 Eastern States and the extent (in those States) of the area covered by the colonization program through 1942 are shown on the accompanying map. Experimental plots involving some 100 acres of turf in 7 States and the District of Columbia, which must also be considered as points from which the disease will spread, are not shown.

Table 1.--Summary of Colonization Work with the Milky Disease Organism
of the Japanese Beetle from 1939 through 1942

| State | Bureau-State program | | | Government reservations | | | Total number of-- | | |
|----------------------|--------------------------|-------------------------|------------------|-------------------------|--|------------------|-------------------|--|--|
| | Number of counties | Approximate number of-- | | Number of sites | Approximate number of acres treated | Sites treated | Acres treated | Pounds of spore dust used in treatments | |
| | | Colony sites | Acres treated | | | | | | |
| Connecticut | 6 | 1,935 | 1,320 | 2 | 88 | 1,937 | 1,408 | 2,639 | |
| Delaware | 3 | 3,962 | 1,981 | 1 | 90 | 3,963 | 2,071 | 4,694 | |
| District of Columbia | -- | 4 | 4 | 40 | 1,889 | 44 | 1,893 | 2,254 | |
| Massachusetts | 1 | 6 | 6 | -- | -- | 6 | 6 | 10.5 | |
| Maryland | 23 | 35,131 | 20,751 | 9 | 1,238 | 35,140 | 21,989 | 44,796 | |
| New Jersey | 20 | 451 | 323 | 10 | 495 | 461 | 818 | 1,041.5 | |
| New York | 17 | 634 | 423 | 15 | 1,683 | 649 | 2,106 | 3,046 | |
| North Carolina | 3 | 10 | 310 | -- | -- | 10 | 310 | 697 | |
| Ohio | 7 | 138 | 146 | -- | -- | 138 | 146 | 789 | |
| Pennsylvania | 22 | 2,234 | 1,570 | 4 | 356 | 2,238 | 1,926 | 3,052 | |
| Rhode Island | 3 | 27 | 40 | -- | -- | 27 | 40 | 84 | |
| Virginia | 12 | 742 | 413 | 10 | 372 | 752 | 785 | 1,016 | |
| West Virginia | 4 | 8 | 5 | -- | -- | 8 | 5 | 13 | |
| Totals | 121 | 45,282 | 27,292 | 91 | 6,211 | 45,373 | 33,503 | 64,132 | |



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